

Poly(ADP-ribose) Polymerase-1 Inhibition Prevents Eosinophil Recruitment by Modulating Th2 Cytokines in a Murine Model of Allergic Airway Inflammation: A Potential Specific Effect on IL-5¹

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We recently used a murine model of allergic airway inflammation to show that poly(ADP-ribose) polymerase-1 (PARP-1) plays an important role in the pathogenesis of asthma-related lung inflammation. In this study, we show that PARP-1 inhibition, by a novel inhibitor (TIQ-A) or by gene deletion, prevented eosinophilic infiltration into the airways of OVA-challenged mice. Such impairment of eosinophil recruitment appeared to take place after IgE production. OVA challenge of wild-type mice resulted in a significant increase in IL-4, IL-5, IL-10, IL-13, and GM-CSF secretions. Although IL-4 production was moderately affected in OVA-challenged PARP-1^{-/-} mice, the production of IL-5, IL-10, IL-13, and GM-CSF was completely inhibited in ex vivo OVA-challenged lung cells derived from these animals. A single TIQ-A injection before OVA challenge in wild-type mice mimicked the latter effects. The marked effect PARP-1 inhibition exerted on mucus production corroborated the effects observed on the Th2 response. Although PARP-1 inhibition by gene knockout increased the production of the Th1 cytokines IL-2 and IL-12, the inhibition by TIQ-A exerted no effect on these two cytokines. The failure of lung cells derived from OVA-challenged PARP-1^{-/-} mice to synthesize GM-CSF, a key cytokine in eosinophil recruitment, was reestablished by replenishment of IL-5. Furthermore, intranasal administration of IL-5 restored the impairment of eosinophil recruitment and mucus production in OVA-challenged PARP-1^{-/-} mice. The replenishment of either IL-4 or IgE, however, did not result in such phenotype reversals. Altogether, these results suggest that PARP-1 plays a critical role in eosinophil recruitment by specifically regulating the cascade leading to IL-5 production. *The Journal of Immunology*, 2006, 177: 6489–6496.

Asthma is a Th2 lymphocyte-mediated inflammatory airway disease characterized by pulmonary eosinophilia, increased mucus production by goblet cells, and airway hyperresponsiveness (1–4). The presence of high levels of allergen-specific IgE is a reflection of an aberrant Th2 immune response (5, 6). In recent years an accumulation of data from mice and humans has identified Th2 cytokines IL-4, IL-5, and IL-13 as major contributors to allergy and asthma. In bone marrow, IL-5 is the major cytokine responsible for terminal differentiation of eosinophils (7–9). IL-5 and GM-CSF are responsible for up-regulation, maintenance, and survival of eosinophils in the lungs upon allergen exposure in sensitive subjects (8).

Poly(ADP-ribose) polymerase-1 (PARP-1)⁴ plays an important role in tissue injury in conditions associated with oxidative stress and inflammation. In several pathological situations that involve massive DNA damage, excessive activation of PARP-1 depletes cellular stores of both NAD and its precursor ATP, leading to irreversible cytotoxicity and potential cell death (10–12). In addition to its effects through NAD metabolism on cell and tissue homeostasis, PARP-1 is thought to participate in inflammation by regulating, directly or indirectly, the expression of several inflammatory factors including adhesion molecules, TNF, interleukins, and inducible NO synthase (iNOS) (reviewed in Refs. 13–16).

We have recently shown that PARP-1 is activated upon allergen challenge in an animal model of allergic airway inflammation and its inhibition by an old generation inhibitor (3-aminobenzamide) or by gene knockout prevented infiltration of inflammatory cells into the lungs upon allergen exposure (17). We have correlated such effects to a modulation of iNOS expression, NF- κ B activation, and IL-8 gene expression. Given that eosinophils are thought to be principal inflammatory cells in the pathophysiology of asthma, through the release of lipid mediators, cytokines, and cytotoxic proteins, we investigated in the present study the role PARP-1 may play in the process of eosinophil recruitment into lungs using an experimental model of allergic airway inflammation. We have focused on key factors underlying eosinophil recruitment namely, the production of Th2 cytokines and IgE as well as mucus production, an important consequence of such recruitment. We used

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⁴ Abbreviations used in this paper: PARP-1, poly(ADP-ribose) polymerase; TIQ-A, thieno[2,3-c]isoquinolin-5-one; iNOS, inducible NO synthase; PAS, periodic acid-Schiff; BAL, bronchoalveolar lavage.

both a genetic approach consisting of PARP-1^{-/-} mice on a homogeneous C57BL/6 genetic background and a pharmacological approach through the use of the novel PARP-1 inhibitor thieno[2,3-c]isoquinolin-5-one (TIQ-A). TIQ-A was recently shown to markedly reduce the extent of brain damage induced by focal ischemia as well as to provide long-term protection against both myocardial reperfusion injury and chronic colitis in rodents (11, 18). Our results clearly show that PARP-1 is involved in allergen-induced lung inflammation by contributing to the process of eosinophil recruitment. Such involvement may be mediated by a specific participation in the cascade leading to IL-5 production. These results suggest a close relationship between PARP-1 and IL-5 and demonstrate that PARP-1 inhibition prevents allergen-induced recruitment of eosinophils upon allergen exposure by modulating the production of IL-5 in a potentially specific manner.

Materials and Methods

Animals

Mice were bred in a specific-pathogen free facility at Louisiana State University Health Sciences Center and allowed unlimited access to sterilized chow and water. Maintenance, experimental protocols, and procedures were all approved by the Louisiana State University Health Sciences Center Animal Care and Use Committee. C57BL/6 PARP-1^{-/-} mice were generated by backcrossing the knockout mice under C57BL/6 × SV129 mixed background obtained from Dr. Z.-Q. Wang (via Dr. M. Smulson, Georgetown University, Washington, DC) with C57BL/6 wild-type mice (The Jackson Laboratory) for at least seven generations. The last generation was interbred to generate the C57BL/6 PARP-1^{-/-} mice.

Protocols for sensitization, challenge, and intranasal administration

Six- to 8-wk-old C57BL/6 wild-type or PARP-1^{-/-} mice were sensitized with i.p. injections of 100 µg of grade V chicken OVA (Sigma-Aldrich) mixed with 2 mg of aluminum hydroxide in saline once a week for 2 consecutive weeks, followed by a challenge with aerosolized OVA 1 wk after the second sensitization. The mice were challenged by placing them in groups of two in a Plexiglas chamber (14 × 14 × 9 cm) and exposing them for 30 min to aerosolized OVA (3% OVA in saline). The OVA aerosol was generated by a Bennett nebulizer. A group of wild-type mice received an i.p. injection of 6 mg/kg TIQ-A (Sigma-Aldrich) 2 h prior to challenge. Control groups were not sensitized or challenged. The mice used in each experiment were of the same litter or the same family. For phenotype reversal, OVA-sensitized PARP-1^{-/-} mice were challenged with OVA as described. Twenty-four hours later, mice received an intranasal administration of either 0.5 µg/50 µl recombinant mouse IL-5 (BD Pharmingen), IL-4 (BD Biosciences), OVA-specific IgE (Serotec), or BSA. Mice were then left to recover and sacrificed 24 h later for bronchoalveolar lavage (BAL) or lung fixations and processing for histological analysis.

Organ recovery and staining

Animals were killed by CO₂ asphyxiation and lungs were fixed with formalin for histological analysis, subjected to BAL, or collected for homogenization to prepare cell suspension for cytokine or IgE assessment. Formalin-fixed lungs were sectioned and subjected to H&E or periodic acid-Schiff (PAS) staining using standard protocols. Collected BAL fluids were subjected to cytospin and stained with H&E for the assessment of number and percentage of eosinophils. Spleens were also removed to prepare cell suspension for Th1 cytokine assessment.

Ex vivo challenge and Th2 and Th1 cytokine assessment

Whole lungs and spleens were removed from the different experimental groups after sacrifice. Following the removal of excess fat, lungs and spleens were cut into pieces and gently pushed through a sterile 100 µM strainer into a petri dish containing MEM supplemented with 5 mM HEPES, 100 U/ml penicillin, and 50 µg/ml streptomycin. Splenocytes and lung cells were collected and centrifuged for 10 min at 4°C. The cell pellets were then suspended in 1.5 ml of ammonium chloride lysis buffer and incubated for exactly 1 min to lyse RBC. The process was stopped by adding 10 ml of MEM. Cells were then centrifuged, resuspended in RPMI 1640 medium (Invitrogen Life Technologies) supplemented with 2 mM glutamine, 100 U penicillin/ml and 50 µg/ml streptomycin, 0.5 µM 2-ME, 10 mM HEPES, and 10% FBS (Invitrogen Life Technologies), and counted

using the trypan exclusion method. Cells were seeded in triplicates at a density of 10⁷ cells/ml after which they were either incubated with medium alone or exposed to 200 ng/ml OVA for 72 h. For the phenotype reversal, lung cell suspensions derived from OVA-challenged PARP-1^{-/-} mice were rechallenged with 200 ng/ml OVA in the presence or absence of recombinant mouse IL-5 (BD Pharmingen), IL-4 (BD Biosciences), or OVA-specific IgE (Serotec) at 100 ng/ml. It is noteworthy that in assessing the effect of TIQ-A on the different cytokines, the drug was only administered to animals before challenge but was not added to cell suspensions during incubation with the different agents. The cytokine assessment was conducted using the Bio-Rad Bioplex System for mouse Th1 and Th2 cytokines accordingly to the manufacturer instructions and specifications. IL-13 was quantified using a commercial ELISA kit (R&D Systems).

IgE quantitation

IgE was quantified by a sandwich ELISA. The plates were coated overnight with rabbit anti-OVA Abs (Serotec) at 4°C and then blocked with 1% BSA in PBS for 1 h at 37°C. Samples, along with IgE standards, were prepared and added to the appropriate wells and incubated for 3 h at 4°C. Polyclonal goat anti-IgE Abs, followed by HRP-conjugated rabbit anti-goat Abs, were used to detect bound IgE. 3,3',5,5'-Tetramethylbenzidine (Sigma-Aldrich) was used to develop the plates, and values were determined by an ELISA plate reader at 490 nm.

Data analysis

All data are expressed as the mean ± SD of values from at least six mice per group unless stated otherwise. PRISM software (GraphPad) was used to analyze the differences between experimental groups by one-way ANOVA followed by Dunnett's multiple comparison test.

Results

PARP-1 inhibition, pharmacologically by a novel and potent inhibitor (TIQ-A) or by gene deletion, prevents inflammatory cell infiltration into the airways of OVA-challenged mice

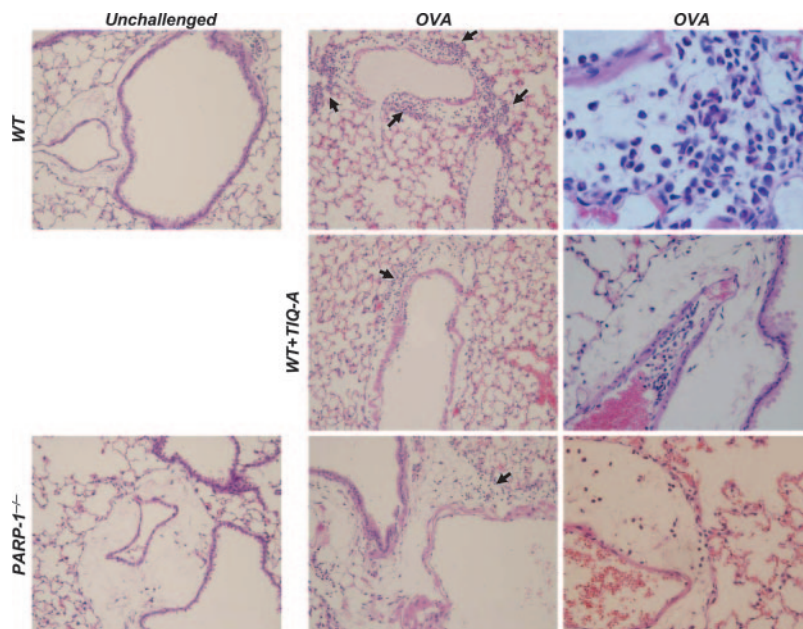
We have recently reported that PARP-1 promotes lung inflammation and that its inhibition prevents infiltration of inflammatory cells into the airways of mice in a mixed C57BL/6 × 129SV genetic background (17). To confirm our observations using an improved method of mouse challenge with OVA, we used PARP-1^{-/-} mice on a C57BL/6 genetic background and TIQ-A, a novel and more potent PARP-1 inhibitor (11, 18). Fig. 1 shows that OVA sensitization and challenge induced a clear and marked perivascular and peribronchial infiltration of eosinophils into the lungs of wild-type mice. Such infiltration of inflammatory cells into the airways of OVA-challenged mice was greatly reduced in wild-type animals that received a single i.p. injection of TIQ-A before challenge. This result was also seen in OVA-challenged PARP-1^{-/-} mice. These results strongly confirm our previous report (17) and further support the notion that PARP-1 may have a critical role in allergen-induced lung inflammation.

PARP-1 promotes recruitment of eosinophils into the lungs of OVA-challenged mice in a post-IgE manner

To determine possible mechanisms by which PARP-1 participates in the recruitment process of inflammatory cells into the airways of challenged mice, we assessed the number of eosinophils in the BAL fluid of mice and examined the specific effects of PARP-1 inhibition on the recruitment of these cells. Fig. 2A shows a marked increase in the number and percentage of eosinophils in the lungs of wild-type mice 48 h after OVA challenge. This effect was reduced considerably in animals that received TIQ-A before OVA challenge and to an even greater extent in the lungs of OVA-challenged PARP-1^{-/-} mice. These results suggest that PARP-1 may play an important role in the process of eosinophil recruitment to the airways following allergen exposure.

We examined whether the marked reduction in eosinophil recruitment into airways of OVA-challenged mice conferred by PARP-1 inhibition was associated with a defect in Ag sensitization

FIGURE 1. Effect of PARP-1 inhibition on inflammatory cell infiltration in a murine model of asthma. C57BL/6 wild-type (WT) or C57BL/6 PARP-1^{-/-} mice were sensitized to OVA and then subjected to a challenge with aerosolized OVA or left unsensitized and unchallenged. Wild-type mice received an i.p. injection (6 mg/kg) of TIQ-A (WT-TIQ-A) or vehicle alone before OVA challenge. Fixed lungs from the different experimental groups were sectioned, stained with H&E, and observed by light microscopy. Magnification for wild-type section, $\times 200$ (left and middle) and $\times 1000$ (right) to show the presence of eosinophils. Magnification for wild-type plus TIQ-A and for PARP-1^{-/-} sections, $\times 400$. Arrows indicate sites of inflammatory cell infiltration. The results are representative of at least four experiments performed at different times.



resulting in a defect in IgE production. Levels of OVA-specific IgE were measured in BAL fluid samples 24 h postchallenge. Fig. 2B shows that PARP-1 inhibition caused a moderate reduction in the levels of OVA-specific IgE. Although statistically significant, these reduction levels did not fully explain the marked diminution in eosinophil recruitment caused by PARP-1 inhibition. Taken together, these results suggest that PARP-1 may not play a major role in the regulation of the cascade leading to IgE expression. Furthermore, the role of PARP-1 in eosinophil recruitment in our model of allergic airway inflammation is downstream of Ag sensitization.

Role of PARP-1 in the production of allergic airway inflammation-associated Th2 cytokines

The expression of Th2-type cytokines is a critical factor in promoting allergen-induced lung inflammation and is crucial for the generation and recruitment of eosinophils into the lung upon allergen exposure (9, 19). Accordingly, we examined the effect of PARP-1 inhibition on the expression of the Th2 cytokines IL-4, IL-5, IL-10, IL-13, and GM-CSF. Cytokine production was measured from lung cells following exposure to OVA as described. As expected, OVA exposure induced the production of each of these cytokines within wild-type mice that were sensitized to and challenged with OVA (Fig. 3). Interestingly, IL-5 and GM-CSF levels were significantly higher than the levels of IL-4, IL-10, and IL-13 in our experimental model. Although the production of IL-5, IL-10, IL-13, and GM-CSF was severely modulated by PARP-1 inhibition, the production of IL-4 was inhibited to a much lower extent. The levels of IL-4 produced by lung cells derived from wild-type mice treated with TIQ-A or from PARP-1^{-/-} mice after OVA exposure were consistent with those of IgE. Thus, IL-4 may not be a major target for regulation by PARP-1. Given that PARP-1 inhibition severely modulated production of IL-5, IL-10, and GM-CSF upon OVA challenge and that these levels are consistent with the marked reduction in eosinophil recruitment, it appears that PARP-1 regulates inflammatory cell infiltration via the activity of these cytokines.

PARP-1 plays an important role in mucus production

Mucus production that occurs as the result of allergen exposure is an important characteristic of the pathogenesis of asthma, and this

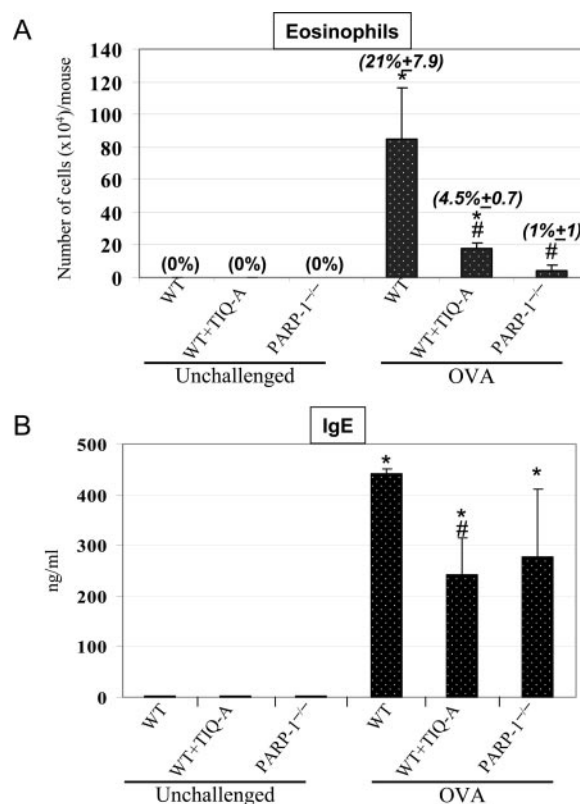


FIGURE 2. Effect of PARP-1 inhibition on eosinophil infiltration into airways and IgE production upon OVA sensitization and challenge. Mice were sensitized to and challenged with aerosolized OVA as described in Fig. 1. Mice were sacrificed 48 h after challenge and then subjected to BAL. A, BAL fluids were collected and centrifuged; cells were then differentially stained, and eosinophils were counted. The percentages at top of the different bars represent the eosinophils from total cell number collected by BAL. Mice were sacrificed 24 h after challenge and then subjected to BAL. B, BAL fluids were tested for IgE levels (nanogram per milliliter) by ELISA. Data are the mean $\pm$ SD of values from at least six mice per group. *, $p < 0.01$ difference from unchallenged mice; #, $p < 0.01$ difference from wild-type mice challenged with OVA.

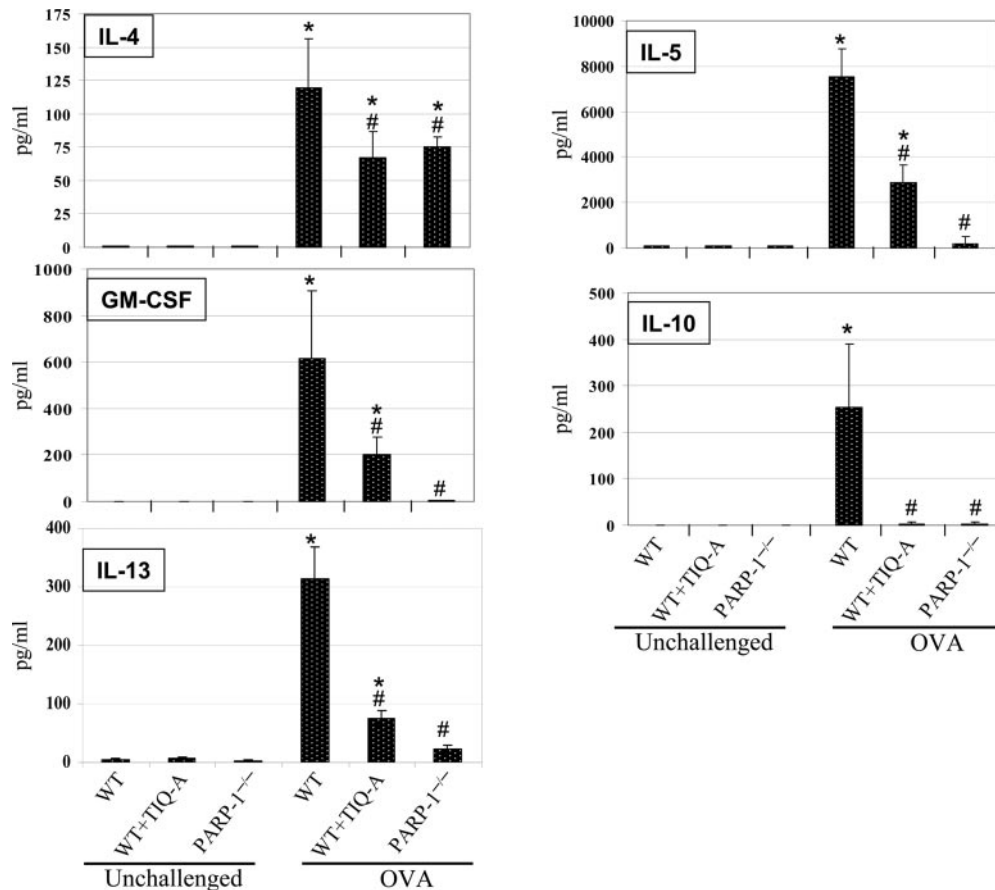


FIGURE 3. Effect of PARP-1 inhibition on Th2 cytokine production following OVA sensitization and challenge. Mice were sensitized to and challenged with OVA or left unchallenged. Mice were sacrificed 24 h after challenge, and their lung cells were isolated. Lung cells from the different experimental groups were incubated with complete medium alone or medium containing 200 ng/ml OVA for 72 h. Cytokine production was measured (picogram per milliliter) using the Bio-Rad Bioplex system or ELISA as described in *Materials and Methods*. Data are given as the mean $\pm$ SD of values obtained from at least six mice per group. *, $p < 0.01$ difference from unchallenged mice; #, $p < 0.01$ difference from wild-type mice challenged with OVA.

production is believed to be highly associated with Th2-type cytokines namely IL-5 and IL-13 (8, 20). To further understand the role of PARP-1 in the inflammatory process after allergen exposure and to explore the function of PARP-1 with the observed Th2 response, we next determined the effect of PARP-1 inhibition on mucus production in our experimental murine model of allergic airway inflammation. Lungs from different experimental mouse groups were stained with PAS to detect mucus-secreting goblet cells. Although lungs of OVA-challenged wild-type mice exhibited substantial mucus production, the lungs of OVA-challenged wild-type mice that received TIQ-A before challenge exhibited a marked decrease in the abundance of PAS-positive goblet cells (Fig. 4). Mucus production was completely blocked in OVA-challenged PARP-1^{-/-} mice. These results are consistent with a role for PARP-1 in the allergen-induced lung inflammatory response via production of Th2 cytokines.

PARP-1 gene deletion up-regulates the production of the Th1 cytokine IL-2 and IL-12 but not IFN- γ

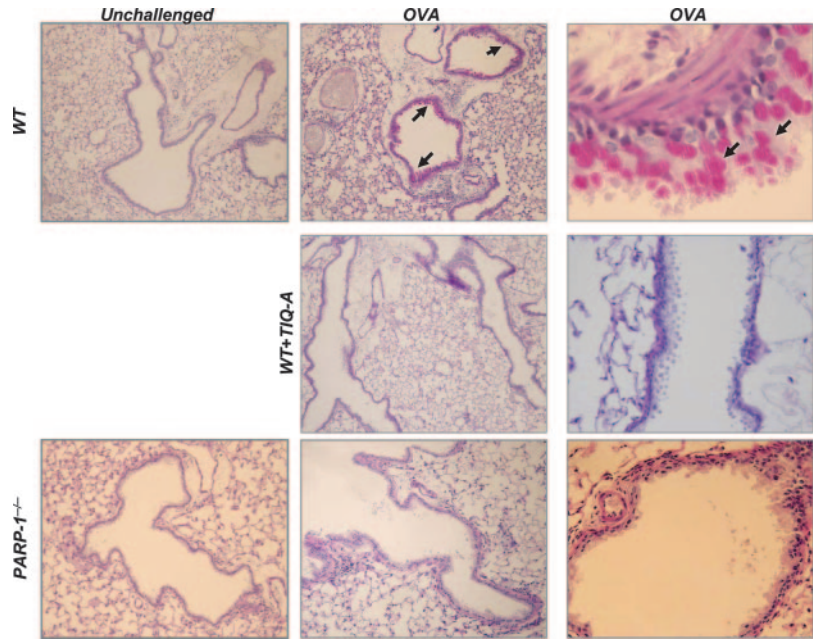
The Th1 cytokines are potent regulators of Th2 cytokines (9, 19, 21, 22). To determine whether PARP-1 inhibition altered the Th1/Th2 balance toward a Th1 response, we assessed the levels of IL-2, IL-12, and IFN- γ in the different experimental groups described in our study. These Th1 cytokines were undetectable in lung cell preparations derived from the different experimental groups that were restimulated, ex vivo, with OVA (data not shown). We next wished to determine whether splenocytes derived from these animals were capable of producing Th1 cytokines. Although PARP-1

gene deletion significantly increased the production of IL-2 and IL-12, PARP-1 inhibition by TIQ-A did not have any significant effect on these two cytokines as their levels remained similar to those in culture supernatants of spleen cells that were derived from OVA-challenged wild-type mice (Fig. 5). In contrast to IL-2 and IL-12, PARP-1 inhibition by either TIQ-A or gene knockout did not increase the levels of IFN- γ in OVA-treated spleen cells. In fact, a slight decrease was observed. These observed discrepancies argue against the possibility that modulation of lung inflammation by PARP-1 inhibition is mediated by an up-regulation of the Th1 response.

Replenishment of IL-5, but not of IL-4 or IgE, reverses the modulating effect of PARP-1 inhibition on OVA challenge-induced GM-CSF production ex vivo and eosinophil infiltration and mucus production in vivo

To ascertain the role of PARP-1 during the process of allergen-induced eosinophil recruitment and to decipher the mechanism by which PARP-1 inhibition modulates this process, we next examined whether an exogenous replenishment of IL-4, IL-5, or IgE would reverse the expression of GM-CSF, a key cytokine for differentiation, proliferation, activation, and survival of eosinophil (19, 23). Lung cells derived from OVA-challenged PARP-1^{-/-} mice were either re-exposed to OVA in the absence or presence of 100 ng/ml IL-5, IL-4, or OVA-specific IgE for 72 h. Following incubation, GM-CSF levels were measured in culture supernatants. Although replenishment of IL-5 conferred the ability to produce

FIGURE 4. Effect of PARP-1 inhibition on mucus production following OVA sensitization and challenge. Mice were sensitized to and challenged with OVA or left unchallenged. Mice were sacrificed 48 h after challenge. Fixed lungs from the different experimental groups were sectioned, stained with PAS, and examined by light microscopy. Magnification for wild-type (WT) sections, $\times 200$ (left and middle) and $\times 1000$ (right) to show the PAS-positive goblet cells. Magnification for wild-type plus TIQ-A (WT+TIQ-a) and PARP-1^{-/-} sections, $\times 400$. Arrows indicate PAS-positive regions. The results are representative of at least three experiments performed at different times.



substantial amounts of GM-CSF to lung cells derived from OVA-challenged PARP-1 mice, replenishment of IL-4 or IgE had little to no effect on the production of this cytokine (Fig. 6A). Similar results were obtained using lung cells derived from OVA-challenged wild-type mice that received TIQ-A before challenge (data not shown). These results clearly suggest that IL-5 may be a critical target for regulation by PARP-1. Furthermore, following

allergen challenge, PARP-1 inhibition may be reducing lung inflammation and recruitment of eosinophils by interfering with the cascade leading to IL-5 production.

A close relationship between IL-4 and IL-5 has been established, and in fact, a reduction in IL-4 production negatively affects the expression of IL-5 (24–26). To further investigate the relationship between PARP-1 and IL-5, we examined the effect of IL-4

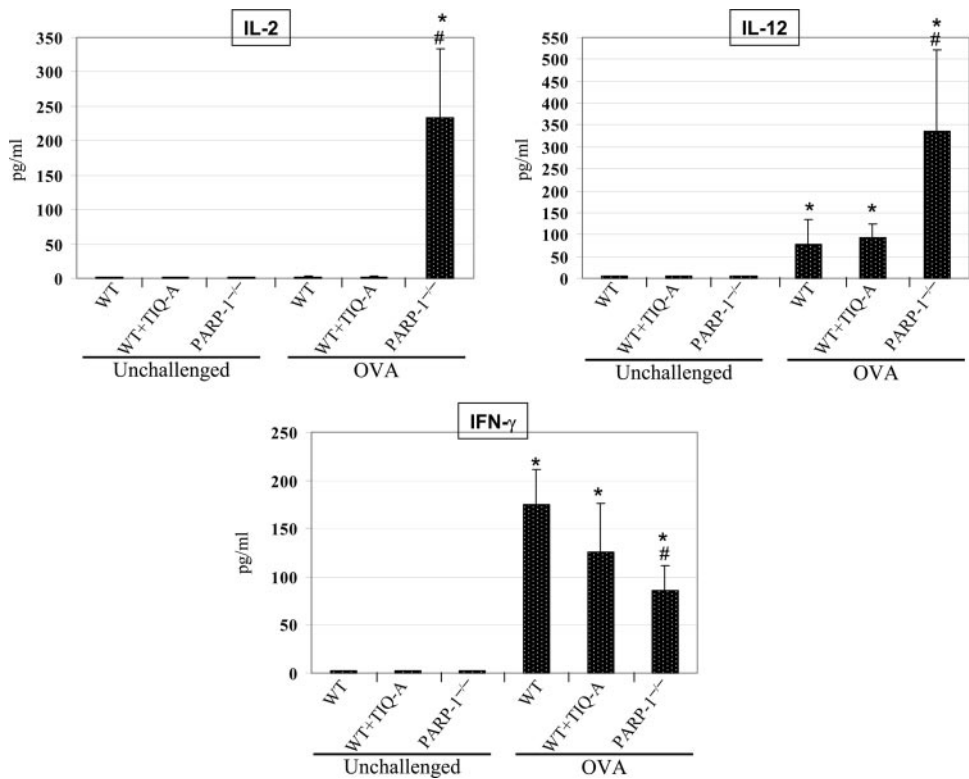


FIGURE 5. Effect of PARP-1 inhibition on Th1 cytokines production following OVA sensitization and challenge. Mice were sensitized to and challenged with OVA or left unchallenged. Mice were then sacrificed 24 h after challenge, and spleen cells were isolated. Spleen cells from the different experimental groups were incubated in complete medium alone or medium containing 200 ng/ml OVA as described in *Materials and Methods*. Cytokine production (picogram per milliliter) was measured using the Bio-Rad Bioplex system. Data are given as the mean $\pm$ SD of values from at least six mice per group. *, $p < 0.01$ difference from unchallenged mice; #, $p < 0.01$ difference from wild-type mice challenged with OVA.

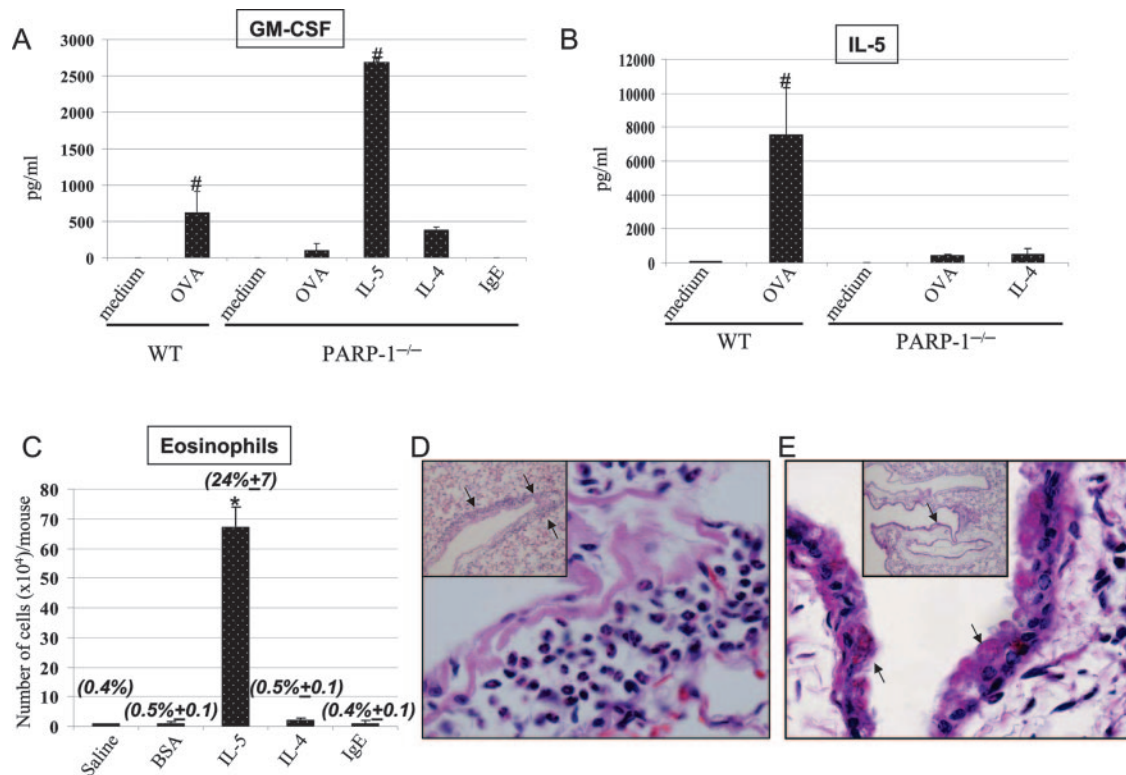


FIGURE 6. Effects of IL-5, IL-4, or IgE replenishment on GM-CSF production by PARP-1^{-/-} lung cells and on eosinophil-recruitment into airways of OVA-sensitized and OVA-challenged PARP-1^{-/-} mice. C57BL/6 PARP-1^{-/-} mice were sensitized to and challenged with OVA. Lung cells were isolated 24 h after challenge and then incubated with OVA in the absence or presence of IL-5, IL-4, or IgE for 72 h. Control cells were incubated in complete medium alone. **A**, GM-CSF expression (picogram per milliliter) was measured using the Bio-Rad Bioplex system. **B**, Effect of IL-4 replenishment on IL-5 production by lung cells derived from OVA-sensitized and challenged PARP-1^{-/-} mice. Lung cells were incubated with OVA or IL-4 or left untreated. IL-5 production was measured (picogram per milliliter) after a 72-h incubation. Data are given as the mean $\pm$ SD of values from at least six mice per group. [#], $p < 0.01$ difference from lung cells derived from OVA-challenged PARP-1^{-/-} mice that were restimulated with OVA in vitro. **C–E**, PARP-1^{-/-} mice were sensitized to and challenged with OVA. Twenty-four hours later, mice were subjected to intranasal administration of recombinant mouse IL-5, IL-4, or OVA-specific IgE. Mice were sacrificed 48 h after OVA challenge. The mice were then subjected to BAL or their lungs were fixed and analyzed histologically. **C**, Eosinophils were counted in BAL fluid. Data are given as the mean $\pm$ SD of values from at least five mice per group. Percentages shown on top of the different bars represent eosinophils from the total cell number collected by BAL. ^{*}, $p < 0.01$ difference from OVA-challenged PARP-1^{-/-} mice that received an intranasal administration of BSA. Fixed lungs from IL-5-treated OVA-challenged PARP-1^{-/-} mice were sectioned and then subjected to H&E (**D**) or PAS (**E**) staining. Sections were then observed by light microscopy. Magnification, $\times 1000$ with a $\times 100$ view (inset). Arrows indicate sites of inflammatory cell infiltration or PAS-positive goblet cells.

treatment of lung cells derived from OVA-challenged PARP-1^{-/-} mice on IL-5 production. IL-4 treatment did not markedly induce IL-5 production (Fig. 6B) clearly suggesting that the cross-talk between IL-4 and IL-5 is affected by PARP-1 inhibition. This result further supports the idea that PARP-1 plays a specific role in the cascade leading to IL-5 production.

Given that replenishment of IL-5 reversed the inhibitory phenotype on GM-CSF by PARP-1 inhibition, we hypothesized that an administration of IL-5 into lungs of OVA-sensitized PARP-1^{-/-} mice after challenge would restore eosinophil infiltration and mucus production. Accordingly, PARP-1^{-/-} mice were sensitized to and challenged with OVA as described. Twenty-four hours post-challenge, the mice received, intranasally, 0.5 μ g/50 μ l recombinant mouse IL-5, IL-4, or OVA-specific IgE and were then sacrificed 24 h later. The lungs of the mice were then subjected to either BAL or formalin fixation followed by histological examination. Although IL-5 replenishment promoted a pronounced eosinophilic infiltration of the airways of PARP-1^{-/-} mice, no such effect was observed with either IL-4 or IgE replenishment (Fig. 6C). Such IL-5-mediated eosinophilic infiltration was predominantly perivascular and slightly peribronchial (Fig. 6D). PAS staining of the histological sections prepared from lungs of IL-5-treated

OVA-challenged PARP-1^{-/-} mice, shows a number of PAS-positive goblet cells indicating mucus production (Fig. 6E). These findings are consistent with the previous results of the effect of IL-5 replenishment on GM-CSF in PARP-1^{-/-} lung cells (Fig. 6, A and B). These results clearly establish a role for PARP-1 in the pathogenesis of OVA-induced lung inflammation in our murine model of allergic airway inflammation and also describe a potentially important regulatory relationship between PARP-1 and IL-5.

Discussion

We have recently shown that PARP-1 may play an important role during allergen-induced lung inflammation (17). We demonstrated that PARP-1 is activated upon allergen challenge possibly through a generation of nitrogen species by iNOS. The activation of PARP-1 during allergen-induced lung inflammation was recently confirmed in a guinea pig model of asthma (27). In this study, we provide novel evidence suggesting a specific role for PARP-1 in the cascade leading to eosinophil recruitment upon allergen challenge and present a possible mechanism by which PARP-1 inhibition prevents such recruitment. Our work suggests that PARP-1

may play an important role in the process of allergic lung inflammation-associated Th2 response by potentially regulating IL-5 production in a specific manner. The inability of lung cells derived from OVA-challenged PARP-1^{-/-} mice to synthesize GM-CSF, a key cytokine in eosinophil recruitment, was reestablished by replenishment of IL-5. More notably, the impairment in both eosinophil infiltration into the airways of OVA-challenged PARP-1^{-/-} mice and mucus production were reversed by an intranasal administration of recombinant mouse IL-5. The addition of IL-4 or IgE caused no such reversal of phenotype.

Asthma is a rather complex disease that involves a variety of cell and noncell components (1, 2, 28). Ag exposure initiates this disease in sensitive subjects and results in an array of events leading to the generation of CD4⁺ Th2 cells, IgE production, recruitment of eosinophils into the airways, hyperresponsiveness, and hyperplasia of goblet cell and the ensuing mucus production (9, 29). The CD4⁺ Th2 cells play a pivotal role in orchestrating the allergic response including effects on the production of key Th2 cytokines necessary for the build up of eosinophils in the lungs as well as activation of B lymphocytes to generate IgE (9, 23). Our data show that PARP-1 inhibition severely blocked the manifestation of eosinophil infiltration and mucus production. This demonstration is the first on the involvement of PARP-1 in the process of eosinophil recruitment, although most reports have shown an effect on neutrophil infiltration in a variety of animal models (16, 30–33). This novel finding is particularly important because recruitment of eosinophils differs greatly from that of neutrophils in terms of the mechanism of the recruitment process as well as the disease state for which they are associated (34).

IL-4 is required for the generation of CD4⁺ Th2 cells and the activation of B cells to induce the production of Ag-specific IgE (22, 25). In our experimental model, PARP-1 inhibition moderately inhibited IL-4 production, which correlated with the effects observed on IgE production. Thus, it is tempting to speculate that the effects on IL-4 production may indirectly mediate the extent of IgE reduction by PARP-1 inhibition after OVA exposure. Importantly, the effects of PARP-1 inhibition on IL-4 and IgE levels do not explain the severe lack of eosinophil recruitment observed in OVA-challenged TIQ-A-treated wild-type or PARP-1-deficient mice. From these results, we speculate that PARP-1 may act downstream perhaps by affecting the production of IL-5 and GM-CSF.

Previous studies have clearly demonstrated that IL-5 and GM-CSF are responsible for up-regulation, maturation, recruitment, and survival of eosinophils during asthma (8, 19). Our findings show that PARP-1 inhibition strongly alters the expression of both IL-5 and GM-CSF. Such modulation correlates with PARP-1 inhibition on eosinophil recruitment in our experimental model. Because IL-5 is the major maturation and differentiation factor for eosinophils and may also be responsible for the activation and degranulation of these inflammatory cells during asthma (8, 9, 34), we have primarily focused our research on the relationship between PARP-1 and IL-5. Recently, Tavernier et al. (35) have shown that only IL-5, not GM-CSF or IL-13, up-regulates the α subunit of the IL-5R and that human eosinophil development is largely IL-5 dependent. In addition, the IL-5R is mainly expressed on eosinophils and to a lower extent on basophils; however, the receptor for GM-CSF is expressed on a wide variety of immune cells (19, 22). Furthermore, our present results show that GM-CSF can be induced by IL-5 in lung cells derived from OVA-challenged PARP-1^{-/-} mice (Fig. 6). This result suggests that the cascade, post-IL-5 production, leading to GM-CSF expression is not defective in PARP-1^{-/-} cells. Indeed, we attempted to reverse this phenotype in lung cells derived from PARP-1^{-/-} mice by stimulating them with IgE, IL-4, or IL-5 and assessing GM-CSF levels. Only

IL-5 was able to induce substantial amounts of GM-CSF indicating that IL-5 is an important candidate for regulation by PARP-1. Whether IL-5 directly induces the expression of GM-CSF or requires additional players in our experimental system is unclear; we are currently pursuing this issue in the laboratory in an attempt to determine the specific cell or cells responsible for this effect and decipher the associated signal transduction pathway. IL-5, in conjunction with endothelin-1, has recently been shown to increase expression of IL-13 (36) and plays an important role in the process of mucus production (37, 38). The effect of PARP-1 inhibition on IL-5 and IL-13 is well in keeping with its effect on mucus production. This relationship was further supported by the ability of IL-5 instillation to reverse, although not to the extent observed in OVA-challenged mice, mucus production in OVA-challenged PARP-1^{-/-} mice.

The production of IL-5 and the other Th2 cytokines tested in this study are modulated by an increase in Th1 cytokines such as IL-2, IL-12, and IFN- γ (5). Our results indicate that IL-2 and IL-12 increased only in spleen cells derived from OVA-challenged PARP-1^{-/-} mice but not in those from OVA-challenged TIQ-A-treated wild-type mice (Fig. 5). Conversely, the low levels of IFN- γ observed in spleen cells derived from OVA-challenged wild-type mice were further diminished after PARP-1 inhibition (Fig. 5). These results argue against PARP-1 inhibition-induced up-regulation of the Th1 response to counteract IL-5 and GM-CSF production and ultimately to inhibit eosinophil recruitment. These findings strengthen the specific relationship between PARP-1 and IL-5.

Although the role of IL-10 in Th2-associated lung inflammation remains unclear and controversial, this protein is increasingly regarded as an anti-inflammatory cytokine (5). Exogenous administration of IL-10 to OVA-challenged mice in a model similar to ours as well as IL-10^{-/-} mice (39) have been used to study IL-10. PARP-1 inhibition completely abrogated the production of IL-10 upon OVA challenge (Fig. 4). We speculate that such reduction was associated with the observed decrease in airway inflammation. Basically, because little to no inflammation was present when PARP-1 was inhibited, the production of this cytokine was therefore unnecessary.

Altogether, our results show a role for PARP-1 in the process of eosinophil recruitment during allergen-induced lung inflammation and provide evidence for a potential specific relationship between PARP-1 and IL-5.

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Disclosures

The authors have no financial conflict of interest.

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CORRECTIONS

Shushakova, N., G. Eden, M. Dangers, J. Zwirner, J. Menne, F. Gueler, F. C. Luft, H. Haller, and I. Dumler. 2005. The urokinase/urokinase receptor system mediates the IgG immune complex-induced inflammation in lung. *J. Immunol.* 175: 4060–4068.

The fourth author, who provided the clone 20/70 rat anti-mouse IgG Ab for this paper, is retracting his authorship from this article. Dr. Jörg Zwirner was not informed before manuscript submission that he would be included as an author. All authors accept this decision, which is the result of an agreement mediated by the Ombudsman of the German Research Council.

In **References**, Dr. Zwirner's last name is misspelled. The corrected Ref. 23 is shown below.

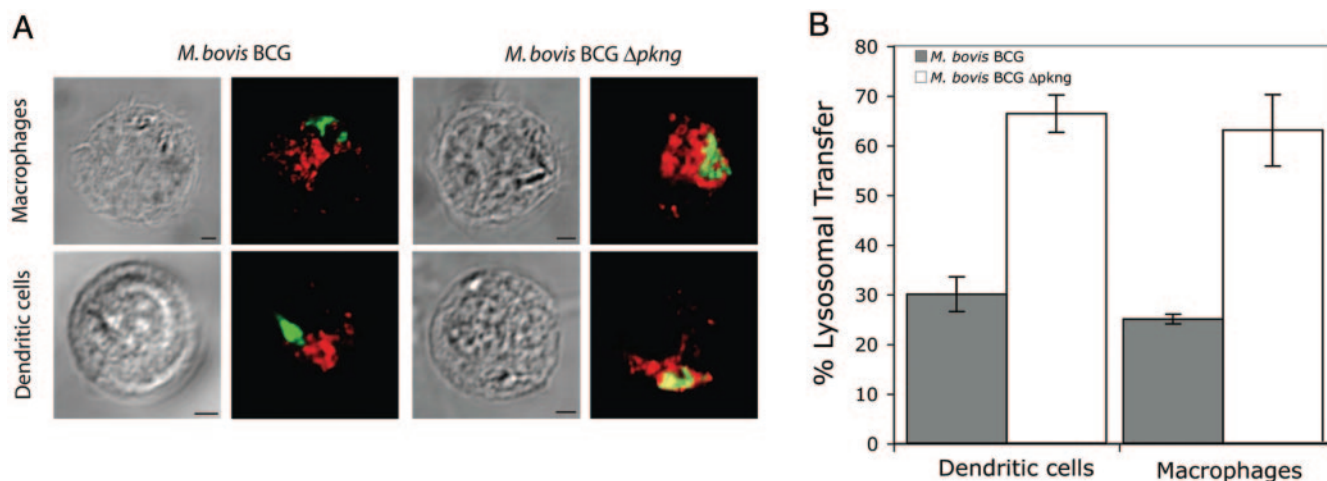
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The first author's first and last names are transposed. The correct name is Mustapha Oumouna.

Majlessi, L., B. Combaluzier, I. Albrecht, J. E. Garcia, C. Nouze, J. Pieters, and C. Leclerc. 2007. Inhibition of phagosome maturation by mycobacteria does not interfere with presentation of mycobacterial Ags by MHC molecules. *J. Immunol.* 179: 1825–1833.

In Fig. 1, dimension bars in A and the symbol key in B were omitted. The two bottom left panels of A are incorrect. The results and conclusions of the paper remain unchanged. The corrected figure is shown below.



Liu, H., R. W. Redline, and Y. W. Han. 2007. *Fusobacterium nucleatum* induces fetal death in mice via stimulation of TLR4-mediated placental inflammatory response. *J. Immunol.* 179: 2501–2508.

In Fig. 4, the labels are missing in the last four panels of B. The corrected figure is shown below.